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DNCB INDUCED CELL MEDIATED RESPONSE IN DMBA TREATED HAMSTER POUCHES

by

Martin Marshack

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of

Master of Science

June

1976

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DEDICATION

To my wife, Arlene
and daughters,
Rosanne
Carla
Sara

ACKNOWLEDGMENTS

My sincerest thanks to Dr. Patrick Toto, Professor and Chairman, Department of Oral and General Pathology, and to Dr. Ronald Kerman, Research Immunologist, Hines Veterans Administration Hospital, who made inception and completion of this project possible. Their unselfish attention to my problems was constant and indeed exemplary.

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BIOGRAPHY

Martin Marshack was born in Chicago, Illinois, on April 2, 1928.

He was graduated from Lane Technical High School, Chicago, on February 2, 1947.

He attended Loyola Dental School, Chicago, Illinois, from 1951 to 1955, subsequent to receiving his Bachelor of Science degree from Roosevelt University in June, 1951. A degree of Doctor of Dental Surgery was conferred on June 7, 1955.

In September of 1974, he entered the Graduate School of Loyola University to begin a two-year program toward a Master of Science Degree in Oral Biology.

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CHAPTER I

INTRODUCTION

The aim of this project was to investigate the possibility of utilizing the immune response of the host to inhibit and/or eliminate oral malignancy. Evidence has shown that malignancies grow and spread often with relatively little, or no demonstrable response by the hosts immune system. These responses may be due to the tumor antigen's lack of strength or inability of the host's innate immune system to recognize the tumor antigen. Another possible concept is that the host's antibodies may cover tumor antigens present on cell surfaces thereby protecting the cancer cells from the cell mediated immune response. This would allow the cancer cells to proliferate and/or metastasize.

In this study dimethylbenzanthracene, DMBA, was used to induce a carcinomatous lesion in the hamster cheek pouch. Dinitrochlorobenzene, DNCB, was employed to sensitize the buccal mucosa prior to, concomitantly with, or after tumor induction of the hamster cheek pouch in order to prevent, to eliminate or inhibit oral pouch tumor development.

Leukoplakia has been recognized as the most common

pre-cancerous lesion of the mouth. The frequency of these premalignant to malignant changes in the oral epithelium can vary from 5 - 7% during an observation period of 3 - 20 years. The hamster cheek pouch model has been successfully used to study this lesion.

The induction of oral cancer in the pouch of the hamster may be achieved by painting the pouch with DMBA in mineral oil.

The progress of this disease begins with erythema, and ulceration followed by repair. Increased mitotic activity, hyperkeratosis with acanthosis, papilloma and finally squamous cell carcinoma are the developmental stages of the lesion.

Practical clinical application of the results of this study could result in sensitizing human leukoplakias by topical application of DNCB or some other immune stimulant. These drugs would immunotherapeutically eliminate the lesion rather than committing the lesion to surgery or risking a wait and watch policy.

CHAPTER II

STATEMENT OF PROBLEM

1. To determine the correct concentration of DNCB which will elicit an optimum cellular mediated response in the hamster cheek pouch.

2. Can DNCB, applied to a hamster pouch mucosa, prevent the induction of tumors by DMBA? Specifically, can varying the time of application of DNCB during tumor induction modify the numbers or sizes of the tumors?

CHAPTER III

REVIEW OF THE LITERATURE

Since this project concerns itself with chemical carcinogens, a brief review of the literature concerning chemically induced tumors was necessary.

Yamigiwa and Ichikawa (1915) induced epithelial malignancy in rabbit ears by cool tar application. Tsutsin, with a similar solution, produced cancer of the skin of mice.

Carcinogenic polycyclic hydro-carbons were described in the early 1930's by Kennaway and Huger. The first, discovered in 1930, was 1,2,5,6 dibenzanthracene, and soon after 3,4-benzpyrene was isolated; 20 methylcholanthrene, a very active carcinogen, was synthesized from bile acids.

Intensified studies have been done on these hydrocarbons; they have been called universal carcinogens because they produce neoplastic disease by topical application, intramuscular and intravenous injection, or by simple feedings (Levy, 1963).

Levy and Ring produced sarcomas in hamsters by subgingival implantation of crystalline 9,10-dimethyl-1,2-benzanthracine. At four to five months, 80% of the

animals had tumors (cited by Salley, 1954). The effect of a single topical application of 9,10-dimethyl-1,2-benzanthracine on the skin and mucous membrane of mouse lips was studied by Levy, Gorlin, and Gottzegen in 1950. After 35 days, some premalignant changes were noted on the labial mucous membrane.

Wantland studied the effect of spraying and painting 20-methyl-cholanthrene, 1-2,5,6-dibenzanthracene, and 2-acetylaminofluorine on the hamster cheek pouch. After six weeks there were no changes other than hyperplasia (Salley, 1954).

Salley (1954) undertook an intensive study to determine the susceptibility of the hamster cheek pouch to carcinogen application, and to find the most suitable carcinogen for this purpose. Thirty-six three month old hamsters were used. The right pouch of each hamster was painted three times a week for sixteen weeks with a carcinogen with a #4 camel hair brush. An additional nine weeks of observation followed this period of painting. Three carcinogens were used in various combinations with two solvents. These potent carcinogens were chosen on the basis of studies by Berenblum and Iball (cited by Salley, 1954). The above information is best shown in the following study (Page 6).

Another group of animals was exposed to acetone

CARCINOGEN-SOLVENT STUDY

Series	Group	Carcinogen	Solvent	No. Animals
A.	I.	9,10-dimethyl-1,2-benzanthracene (DMBA)	Acetone	3 Female 3 Male
	II.	20-methylcholanthrene	Acetone	3 Female 3 Male
	III.	3,4-benzpyrene	Acetone	3 Female 3 Male
B.	IV.	9,10-dimethyl-1,2-benzanthracene	Benzene	3 Female 3 Male
	V.	20-methylcholanthrene	Benzene	3 Female 3 Male
	VI.	3,4-benypyrene	Benzene	3 Female 3 Male

and benzine applications to determine the effects of the solvents listed in the preceding study. Another group was painted with physiologic saline to study any possible traumatic effects of the brushing. The animals were examined and weighed weekly. After sacrificing the animals with chloroform, whole pouches were dissected out and fixed with Bown's fluid. After dehydration and embedding, several sections were cut and stained with hematoxylin and eosin and Mallory's connective tissue stain. Salley also sectioned cervical lymph nodes, tongue, palate, eosophagus, fore stomach, stomach, lungs, and liver.

The first tissue changes were noted in the group using only benzene. Acute inflammation with necroses and sloughing were evident after two weeks. Adhesions had occurred in the pouch, reducing the normal three to three and one-half centimeters pouch to one and one-half centimeters. In this same period of time, six hamsters of group B died; two hamsters of the benzene control group also died. Survivors of these groups healed with no recurrence of inflammation.

Microscopic examination revealed four levels of change: 1) hyperplasia; 2) benign papilloma; 3) squamous carcinoma <u>in situ</u> (pre-invasive); and 4) squamous cell carcinoma with local invasion and metastasis. The

following chart shows the results obtained (Page 9).

There were six original animals in each series. Because of the high mortality rate, benzene proved unsatisfactory. This is in agreement with Bradbury, Bachman, and Lewisohn. Bradbury, Bachman, Lewisohn, Stowal, and Cramer also found acetone very satisfactory as a solvent. It's free miscibility with water allows the carcinogen to be transported into the cell with ease (Salley, 1954).

A glance at the following chart shows that 9,10-dimethyl-1,2-benzanthracene (DMBA) in acetone is the most potent, effective carcinogen. Mineral oil replaced acetone as a more effective vehicle which will be cited further in this paper. Later, Morris (1961) tested the optimum concentrations of DMBA in the hamster pouch. He found that 1.5% solution was extremely toxic and caused a high percentage of mortality before initial lesions developed. Even the survivors required the same length of time to develop tumors as the 0.5% group. Morris found a 0.1% solution increased the latent period (period required to find some evidence of tumor), so that its use was not practical. Thus Morris found, as Salley did, that 0.5% was an optimum concentration of DMBA for producing tumors.

Morris (1961) also used this same carcinogen and painted animals both two and three times a week. Three

ACETONE SERIES

	Number of Survivors	Number With Benign Tumors	Number With Carcinoma	Number With Metastasis	Time of Onset (Weeks)
DMBA	5	0	5	5	7
20-Methyl-cholanthrene	5	2	2	0	25
3,4-Benzpyrene	5	1	2	2	16

BENZENE SERIES

DMBA	4	0	3	3	8
20-Methyl-cholanthrene	3	1	0	0	25
3,4-Benzpyrene	4	0	0	0	--

times a week was found most satisfactory, as Salley had previously determined. A shorter latent period was found for the latter schedule.

Morris (1961) also studied the effect of age and sex on hamsters receiving DMBA applications to the pouch. Starting animals at three weeks, six weeks or nine weeks of age made no difference in response to the carcinogen. Animals one year and a half, and older, showed increased resistance to tumor induction. The sex of the specimen had no influence on the final results. In his experiment, using 0.5% DMBA applied three times a week to the hamster pouch, Morris obtained tumors in most animals after 35 paintings or 12 weeks. Morris tested the various aspects of Salley's experiments and found a 0.5% carcinogen, concentration method of application, and a number of applications to be optimal for tumor production in the hamster cheek pouch.

In another study, Salley (1955) improved his method for acquiring tumors in the hamster cheek pouch. The acetone solvent was replaced by a nonvolatile mineral oil (U.S.P. heavy) solvent. This modification of solvent decreased induction time with tri-weekly applications of DMBA from 6 to 7 weeks (with acetone as a solvent) to 4 1/2 weeks. Fifty weeks of mineral oil paintings, per se, caused no carcinogenic activity.

Renstrup, et al. (1962) demonstrated that chronic mechanical irritation by use of producing ulceration in the cheek pouch of the hamster increases the onset of cancer induced by DMBA.

Morris and Reisken (1965) showed that when the hamster cheek pouch was exposed to tri-weekly applications of DMBA for four weeks, all animals developed tumors in a minimum amount of time. Painting less frequently than three times a week, failed to produce tumors in all animals in twenty-one weeks.

Elzay (1966) studied hamster pouches painted with DMBA in alcohol and noted that clinically they developed epithelial tumors earlier and larger than those painted with DMBA in mineral oil.

Reisken and Berey (1968) showed that carcinomas induced in the pouches of eight to twelve week-old hamsters by DMBA differed in behavior according to the host strain. The mean latent period in inbred dark-eared partial albino (DEA) hamsters was 7.3 weeks, compared to 10.0 to 10.75 weeks in random-bred golden or cream hamsters. The average tumor growth rate was significantly higher in DEA animals than in the other two strains.

Duncan (1969) studied the relation of certain hydrocarbons to metabolism and binding to cellular macromolecules. He found that the potent carcinogens were to

bind DNA and RNA ten times more readily than non-carcinogens.

Immunotherapy

The demonstration of tumor specific antigens in human cancers was evidence that immunotherapy was an important mode of treatment. Evidence accumulated suggested that the cancer patient's immune response to these tumor specific antigens was important in controlling the development and progression of their disease. Those patients who can demonstrate an active immune system have a better prognosis than those with general depression of their immune reactivity. These facts were cited by Hersh in 1971.

Klein has shown that induction of delayed hypersensitivity reaction at tumor sites resulted in selective reactions against premalignant and malignant epidermal lesions which led to their eradication (1969).

Levis et al. showed 70% of basal cell carcinomas treated with DNCB underwent complete regression (1973).

Klein believed the induction of delayed hypersensitivity reactions in tumor nodules or tumor tissues resulted in tumor regression in patients with basal or squamous cell carcinomas of the skin. Klein's initial immunotherapeutic approaches were introduced to patients with primary epidermal neoplasms. These neoplasms included

multiple, extensive, premalignant epidermal lesions such as keratosis of the surface epidermis and leukoplakia involving mucous surfaces and mucocutaneous structures. Basal cell carcinomas, and squamous cell carcinomas, were also studied.

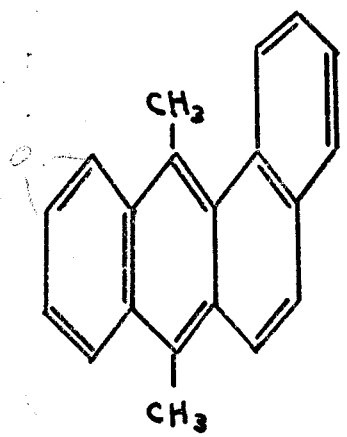
The disappearance of locally invasive human cancer following therapeutic imposition of a controlled allergic contact dermatitis provided a useful model for the study of immunotherapy of cancer. This study applied the principle of induced sensitivity and the induction of squamous cell carcinoma as a model employing the hamster cheek pouch.

CHAPTER IV

MATERIALS AND METHODS

A. Tumor Producing Agent

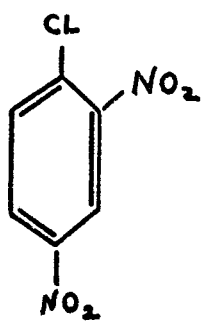
The carcinogen chosen for this study was 9,10 dimethyl 1,2, benzanthracene (DMBA).



This carcinogen has been used by Salley and others to produce tumors in the hamster cheek pouch (Figure 1). A #4 camel hair brush was used to paint a 0.5% solution of DMBA in heavy mineral oil into the cheek pouch. There is 15 mg. of DMBA in one application.

B. Sensitizing Agent

Dinitrochlorobenzene (DNCB)



A yellow crystalline substance was used as a reagent for the detection and determination of nicotinic acid, nicotinamide and other pyridine compounds; a powerful skin irritant which resulted in a dermatitis. Two pellets of cotton containing the solution of DNCB were introduced deeply into the pouch and left to remain until expelled by the hamster, usually after ten minutes. Each saturated pellet of cotton contained approximately one ml. of DNCB in acetone.

C. Animals

Thirty-seven three month-old dark-eared albino (DEA) hamsters were used. The animals were fed Purina rat chow and tap water. Animal immobilization was accomplished by grasping the animals in a gloved hand and exposing the pouch by introducing a looped wire fastened to a stand, into the corner of the mouth (Figure 2).

D. Cell Mediated Response Determination

The objective was to determine an optimum concentration of DNCB which would elicit a cell mediated response without injury to the animal or pouch mucosa as observed clinically. Concentrations of 0.1%, 0.5%, 1%, 5% and 10% DNCB in reagent grade acetone were administered to six hamsters. Two pellets of cotton, each containing 1 ml. of the various concentrations of DNCB were introduced deeply into each pouch and left to remain until

expelled by the hamster, usually after ten minutes (Table I).

Two hamsters received 0.1% solutions in their left pouch and 0.5% solutions in their right pouch. Two hamsters received 1% solutions in their left pouch and 5% solutions in their right pouch. The last two hamsters received a 10% solution in their left pouch and the right pouch was used as a control. Each hamster received the sensitizing doses on days one and three and the mucosa was challenged two weeks from the second sensitizing dose, or day seventeen. The challenging dose was one tenth the concentration of each of the initial sensitizing doses. One week of observation was allowed following the challenging dose for any reaction to occur. At this time the animals were sacrificed with a lethal dose of diethyl ether.

This initial attempt failed in that the solutions proved to be too concentrated. One of the animals treated with 10% DNCB died before the sacrifice day and others showed frank ulcerations through the pouches to the external skin. Other pouches with the exception of the lowest concentrations showed large ulcerated areas (Figure 3).

A second series of dilutions of DNCB was employed (Table II). The new range of solutions were 0.1%, 0.5%, .001%, .0005%, .00001%, .000005% of DNCB in acetone. The

control solution was composed of pure reagent acetone. The seven concentrations were administered to fourteen hamsters according to the following schedule. On the first and third days of the experiments, two hamsters received 0.1% DNCB in each of four pouches. The next two hamsters received .05% DNCB in each of four pouches. The following two hamsters received a .001% DNCB in each of four pouches. The following two hamsters received a .0005% DNCB in each of four pouches. The following two hamsters received a .00001% DNCB in each of four pouches. The following two hamsters received a .000005% DNCB in each of four pouches. The last two hamsters served as controls and received only a solution of acetone in each of four pouches. The hamsters were then allowed to rest for fourteen days. At this time each hamster received the challenging doses of DNCB. One week of observation for adverse tissue reaction was again allowed following the challenging dose. At this time the hamsters were sacrificed with a lethal dose of diethyl ether.

The pouches were removed from the hamsters in all experimental groups, fixed in formation for twenty-four hours, washed in tap water and then they were processed to increasing concentrations of alcohol. The concentrations of alcohol were 65%, 70%, 80%, 95%, and 100%. The pouches were then transferred and cleared in xylene and then

embedded in liquid paraffin. Paraffin blocks were made and six micron sections were cut on a microtome and stained with hematoxylin and eosin.

It was determined that the 0.1% concentration of DNCB produced an optimum cell response characterized by a lymphocytic infiltration in the lamina propria. This concentration was used to sensitize the mucosa in the following DNCB-DMBA hamster pouch tumor induction study.

E. DNCB-DMBA Hamster Pouch Tumor Induction

The following is a schedule employed using DNCB and DMBA in three separate experiments (Tables III A, B, and C).

Experiment I, Group A

This experiment employed three hamsters. A 0.1% DNCB concentration in acetone with approximately 1 ml. of DNCB per pellet was administered to both pouches of three hamsters on day one and day three. Fourteen days later the pouches were challenged with a .01% DNCB concentration in acetone. On day eighteen twice weekly concentrations of 0.5% DMBA in mineral oil were administered by painting with a #4 camel hair brush with approximately 15 mg. of DMBA per application. The twice weekly painting continued for fourteen weeks at which time the animals were sacrificed (Table III A).

Experiment II, Groups B, E, F, G, H

This experiment employed ten hamsters. A 0.1% DNCB concentration was administered to both pouches of ten hamsters on day one and day three and fourteen days later the pouches were challenged with a .01% DNCB concentration. On day eighteen twice weekly paintings of 0.5% DMBA in mineral oil were administered as in experiment I for fourteen weeks. At this time eight of the ten hamsters were rechallenged with the .01% DNCB concentration; two hamsters not challenged with DNCB were used as controls. These two hamsters received only the DNCB and DMBA as in experiment I but no further challenge with DNCB.

One week after the first rechallenge two hamsters were sacrificed. The remaining six hamsters again were rechallenged with a 0.01% DNCB solution applied to their pouches. One week later, two of the six remaining hamsters were sacrificed. The remaining four hamsters were again rechallenged with a 0.01% DNCB solution. One week later two of the four hamsters were sacrificed. The remaining two hamsters again were rechallenged with a 0.01% DNCB solution. In the next and final week the last two hamsters were sacrificed. Also, the two control hamsters were sacrificed at this time (Table III B).

Experiment III, Groups C, D

This experiment employed four hamsters. Unlike

experiment I and II, experiment III begins with first inducing the tumor by twice weekly paintings with 0.5% DMBA in mineral oil for fourteen weeks. Then at this time, three hamsters were treated with the predetermined sensitizing doses of 0.1% DNCB on day one, day three and a challenging dose of .01% DNCB fourteen days later. The three hamsters were then sacrificed.

The fourth hamster was kept as a non-DNCB sensitized control and received only DMBA paintings for fourteen weeks and was then sacrificed. Note: no tumors were counted clinically before or after DNCB administration (Table III C).

The pouches were removed from all sacrificed hamsters, fixed in formalin for twenty-four hours. They were dehydrated in ascending alcohols, cleared in xylene and embedded in paraffin. Sections were cut at six microns and stained with hematoxylin and eosin. The sections from all pouches were examined histologically and the tumors were counted and described. Measurements of the tumor dimensions as seen on the histologic slide were recorded. Also, using a reticular eyepiece containing a 50 micron square grid, the number of lymphocytes and plasma cells per fifty micron square field was averaged from three randomly selected fields and recorded.

CHAPTER V

RESULTS

Experiment III, Group D (Table VI)

This was the control group in which one hamster received DMBA paintings only, for fourteen weeks. Two advanced squamous cell carcinomas were seen, one in each pouch (Figure 4). The two tumors measured 450 microns by 250 microns and 400 microns by 300 microns, respectively. All the classical characteristics of malignancy were present with hyperchromatism, frequent mitotic figures, cell nests and papillary projections. The cellular infiltrate in the connective tissue confronting the tumors was slight, an average of 12.2 lymphocytes per 50 microns^2 was seen and an average of 0.66 plasma cells was seen per 50 microns^2.

Experiment III, Group C (Table VI)

This group represents three animals treated first with DMBA and then treated with DNCB. Twelve non-invasive papillary tumors were seen (Figure 5). The size of the papillomas averaged 142 x 150 mm. apparently smaller than in the pouches in the Experiment III, Group D control. Four pouches were examined. Four tumors of the papilloma type were seen in one pouch, three tumors were observed in

a second and third pouch, and two tumors were seen in a fourth pouch. In this group the cellular infiltrate exhibited an average of 50 lymphocytes/50 microns^2 and 2.7 plasma cells/50 microns^2 (Figure 6). The lymphocytes were seen invading the epithelium as well as the connective tissue confronting the tumor. It was observed that some areas which were non-tumor bearing showed ulceration of the epithelium and, also, exhibited a heavy infiltrate of lymphocytes into the lamina propria and epithelium (Figure 7). Polymorphonuclear leukocytes were also accompanying the lymphocytes and plasma cells (Figure 8).

Experiment I, Group A (Table IV)

This group represents pretreatment with DNCB and then treatment with DMBA. A total of two non-invasive papillary tumors were found in four pouches. One tumor was a carcinoma *in situ* which showed hyperplasia with dyskeratosis, hyperchromatic cells, mitosis and acanthosis. The other tumor was a papillary carcinoma showing similar histologic characteristics. Also, the non-tumor areas of the pouch mucosa showed hyperkeratosis with acanthosis (Figures 9 and 10). The cellular infiltrate showed an average of 20 lymphocytes/50 microns^2 and .5 plasma cells/50 microns^2 as seen under a high power field. This lymphocytic infiltrate was seen invading into the epithelium in ulcerated non-tumor bearing areas (Figure 11).

Experiment II, Groups B, E, F, G, H (Table V)

This group represents animals pretreated with DNCB then treated with DMBA for fourteen weeks and then rechallenged with DNCB. A total of five tumors of the papillary carcinoma type as seen before in the other experiments were found in twenty pouches. Seventeen pouches showed no tumors. The tumors were papillary in nature and on the average measured 70 microns x 100 microns. A few areas of hyperkeratosis with acanthosis were seen with an infiltration of lymphocytes into the lamina propria and epithelium of non-tumor bearing areas (Figure 12). Two control animals in Group H showed two squamous cell carcinomas *in situ* measuring 300 microns x 400 microns. These animals received initial DNCB treatment and subsequent DMBA administration with no rechallenge of DNCB.

CHAPTER VI

DISCUSSION

The use of DNCB in hamster pouches to induce the cell mediated response is positively effective. However, high concentrations of DNCB is injurious to the pouches producing severe ulcerations. DNCB dilutions of 0.1% concentrations in acetone will induce an optimum cellular response without noticeable tissue modification to the hamster pouch. The demonstrated cell mediated response corresponds well to studies undertaken by Klein and Holterman who showed induction of delayed hypersensitivity reactions at tumor sites resulted in reactions against epidermal lesions which led to their eradication. Also, Levis et al. showed basal cell carcinomas treated with DNCB underwent complete regression. This study showed a cell mediated response can be produced in the hamster pouch. Therefore, the concept that the hamster pouch is a privileged site, as reported by Shepro, cannot be supported by this study.

Shepro et al. has pointed out the hamster pouch is an "immunologically privileged site". It therefore is purported to possess poor immunological ability. This concept was based on transplant studies in which skin grafts showed a long survival time in cheek pouch compared to

other parts of the body. A lack of lymphotic drainage has been reported to be the cause of the immunological privileged site. However, Lindemann in 1968 did demonstrate the presence of lymphocytic vessels in the cheek pouch of the hamster.

The hamster pouches develop tumors when treated with DNCB-DMBA. However, hamster pouch tumor induction resulted in the greatest number of tumors when DMBA induction of tumors was followed by DNCB treatment. This was supported by the fact a total of twelve papilloma type non-invasive tumors were seen in four hamster pouches using this regimen. When sensitization with DNCB occurred before the induction of tumors by DMBA, only two non-invasive tumors were found in the four hamster pouches. This treatment schedule using DNCB prior to DMBA tumor induction appeared to be further effective when DNCB challenge of the mucosa bearing tumors was again employed. This became evident as only five non-invasive tumors were seen in twenty pouches.

The hamster pouches treated with DMBA alone, showed two invasive type squamous cell carcinomas in two pouches. This would suggest DNCB did not alter tumor induction. However, it is apparent in the presence of a cell mediated response produced by DNCB administered either before or after DMBA, tumors will form. Treatment with DNCB prior to

DMBA showed reduced tumor numbers. It is significant that invasive tumors were seen only in the DMBA control animal while none was seen in the pouches treated with DNCB.

All of the tumors found in the pouches treated with DNCB were of a smaller size than the tumors induced by DMBA alone.

A lymphocytic and plasma cell mediated response was seen in all DNCB treated groups. In addition, the cell mediated response was greater than the pouches not treated with DNCB. In post-treated DNCB hamsters the cell mediated response was greatest with an average count per 50 microns^2 in a high power field showing 50 lymphocytes and 2.7 plasma cells. This compares with the average count of 20 lymphocytes and .5 plasma cells in animals pretreated with DNCB. In the hamster given DMBA and not sensitized with DNCB, there also occurred a cell mediated response. The average count was 12.2 lymphocytes/50 microns^2 and .66 plasma cells/ 50 microns^2. This suggested hamsters are capable of a cell mediated response without DNCB. Conversely, however, when DNCB treatment was employed the cell mediated response was clearly enhanced.

An important observation is the presence of lymphocytes seen not only in the lamina propria, but also invading the epithelium (Figure 7). It may be significant that the lymphocytes seen in the epithelium resulting from

a cell mediated response are reacting to some tumor associated antigen. To be effective, these lymphocytes must contact the tumor cells in the epithelium to effect an antigen-lymphocyte response. It was significant that a lymphocytic infiltrate was also observed in non-tumor bearing areas of DMBA-DNCB treated mucosa. In such areas microulcers were found. It is a possibility such ulcers may have resulted from a reaction between lymphocytes and epithelial cells containing tumor associated antigens. Such cells may have been part of a preexisting tumor destroyed by the lymphocytes or a reaction with tumor associated antigens on epithelial cells which have undergone neoplastic transformation. Furthermore, the ulcer may have been the result only of DNCB sensitized epithelial cells which were lost as a result of a cell mediated response which occurred upon challenge with DNCB. This suggestion was supported by the studies of Klein and Holterman and Levi who stated tumor progress may be altered by sensitizations and challenge with DNCB. Future studies with an increased number of specimens should be performed to validate the results of this pilot study.

CHAPTER VII

SUMMARY

Thirty-seven three month-old dark-eared albino hamsters were used to determine the capacity of the hamster pouch to raise a cell mediated response.

It also was the intent of the study to determine if DNCB sensitized mucosa either prevented tumor development, reduced tumor number or destroyed the tumor.

This investigation was divided into two sections. The first section was to determine the dilution of DNCB that could raise a cell mediated response without clinically destroying the animal or mucosa. This was found to be 0.1% DNCB in acetone.

The second section of the study consisted of three experiments.

The first experiment employed three hamsters. The hamsters were pretreated with DNCB and then DMBA tumor induction was initiated.

The second experiment employed ten hamsters. The hamsters were pretreated with DNCB and then DMBA tumor initiation followed by weekly challenges with DNCB.

The third experiment employed four hamsters. The hamsters were administered DNCB initially followed by DNCB

post-treatment. A control hamster received only DMBA. All DMBA applications were 0.5% in mineral oil.

All of the animals treated with both DNCB and DMBA developed tumors. However, there was a reduction in tumor size and no tumor invasiveness seen in animals treated with DNCB when compared with animals not treated with DNCB.

The pouch treated with DNCB showed a cell mediated response in all instances. This was noted whether or not a tumor was present. This demonstrated the capacity of DNCB to induce a cell mediated response.

CHAPTER VIII

CONCLUSION

Tumors were induced in hamster pouches with DMBA alone or in combination with DNCB.

The mucosa of the hamster was capable of producing a cell mediated response with a 0.1% DNCB concentration in acetone.

There is suggestive evidence that the tumors induced in hamster pouches are smaller in size when the pouches are treated with DNCB and DMBA than with DMBA alone.

Hamster pouches which form tumors induced by DMBA alone showed a cell mediated response although the number of lymphocytes was less than when treated with DNCB.

DNCB enhanced the cell mediated response in hamster pouch tumors.

CHAPTER IX

BIBLIOGRAPHY

Berenblum, I: Carcinogenesis and Tumor Pathogenesis, Advances in Cancer Research, New York, 2:129-175, 1954, Academic Press, Inc.

Chaudry, A.P.: Carcinogenesis in the Oral Mucosa, (abstract), Journal of Dental Research, 34:750, 1955.

Collins, T.: Experimental Oral Cytology, Partial Fulfillment M.S. Degree, Loyola University, 1972.

Elves, M.: The Lymphocyte and Immune Responses, The Lymphocyte, pages 130-131, 1966.

Elzay, J.: Effects of Alcohol on the Hamster Cheek Pouch, Journal of Dental Research, 45:1792, 1975.

Goldhaber, P.: Further Studies on Experimental Oral Carcinogenesis, Journal of Dental Research, 37:18, 1958.

Goldhaber, P.: The Role of Saliva and Other Local Factors in Oral Carcinogenesis, The Journal of the American Dental Association, 54:517-524, 1957.

Henefer, E.P.: Ethanol, 30 Per Cent, and Hamster Pouch Carcinogenesis, Journal of Dental Research, 45: 838-844, 1966.

Immunosuppression and Malignancy, Editorial, British Medical Journal, 2:713, 1972.

Klein, E. and Holterman, O.A.: Immunotherapeutic Approaches to the Management of Neoplasms, The Journal of the National Cancer Institute Monograph, 35:379, 1973.

Klein, E.: Hypersensitivity Reactions at Tumor Sites, Cancer Research, 29:2351, 1969.

Klein, E.: Tumors of the Skin. X. Immunotherapy of Cutaneous and Mucosal Neoplasms, New York State Journal of Medicine, 68:900, 1968.

Levis, W.R., Kraemer, K.H., Klinger, W.G., Peck, G.L., and Terry, W.D.: Topical Immunotherapy of Basal Cell Carcinomas with Dinitrochlorobenzene, Cancer Research, 33:306, 1973.

Levy, B. and Ring, J.R.: The Experimental Production of Jaw Tumors in Hamsters, Oral Pathology, Oral Surgery, Oral Medicine, 3:262-271, 1950.

Levy, B.M., Gorlin, G., and Gottsegen, R.: A Histologic Study of the Reaction of Skin and Mucous Membrane to a Single Topical Application of the Carcinogen 9,10-Dimethyl-1,2-Benzanthracene, Journal of Dental Research, 29:678-679, 1950.

Levy, B.M.: Experimental Oral Carcinogenesis, Journal of the American Dental Association, 42:321-327, 1963.

Levy, B.M.: The Experimental Production of Oral Malignancies, Journal of the American Dental Association, 54:514-517, 1965.

Morof, A.: Preliminary Study of Hamster Pouch Tumor Antigenicity, Partial Fulfillment M.S. Degree, Loyola University, 1972.

Morris, A.L.: Factors Influencing Experimental Carcinogenesis in the Hamster Cheek Pouch, Journal of Dental Research, 40:3-15, 1961.

Morris, A.L. and Reiskin, A.B.: Hamster Pouch and Varying Carcinogen Exposure, Journal of Dental Research, 44:666-667, 1965.

Reiskin, A.B. and Berry, R.J.: Cell Proliferation and Carcinogenesis in the Hamster Cheek Pouch, Cancer Research, 28(5):898-905, 1968.

Salley, J.J.: Experimental Carcinogenesis in the Cheek Pouch of the Syrian Hamster, Journal of Dental Research, 33:253-262, 1954.

Salley, J.J.: Experimental Carcinogenesis in the Cheek Pouch of the Syrian Hamster, "Thirty-First General Meeting," Journal of Dental Research, 26:679, Vol. 5, 1954.

Salley, J.J.: Effect of Mineral Oil as a Solvent for 9,10-Dimethyl-1,2-Benzanthracene, Journal of Dental Research, 34:723, 1955.

Salley, J.J.: Histologic Changes in the Hamster Cheek Pouch During Early Hydrocarbon Carcinogenesis, Journal of Dental Research, 36:48-55, 1957.

FIGURE 1

DMBA INDUCED TUMOR IN HAMSTER CHEEK POUCH

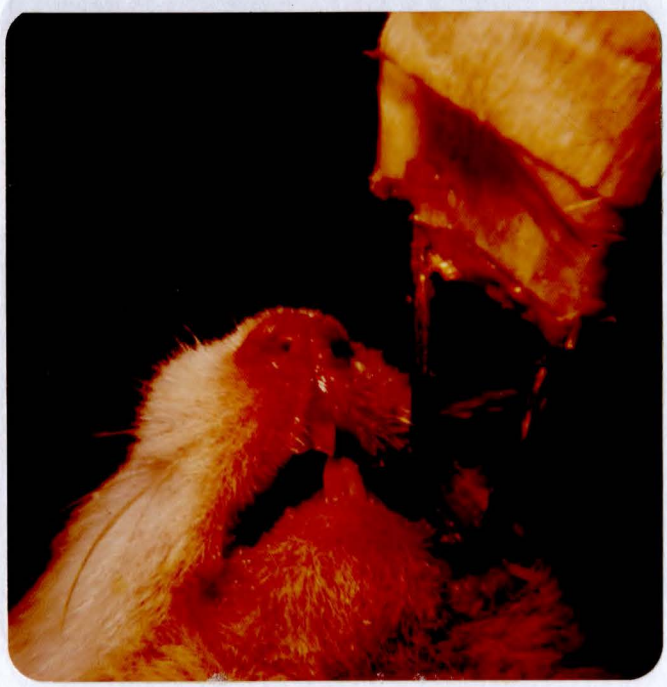


FIGURE 2

EXPOSING HAMSTER CHEEK POUCH BY INTRODUCTION OF LOOPED WIRE

FIGURE 3

ULCERATED CHEEK POUCH TISSUE

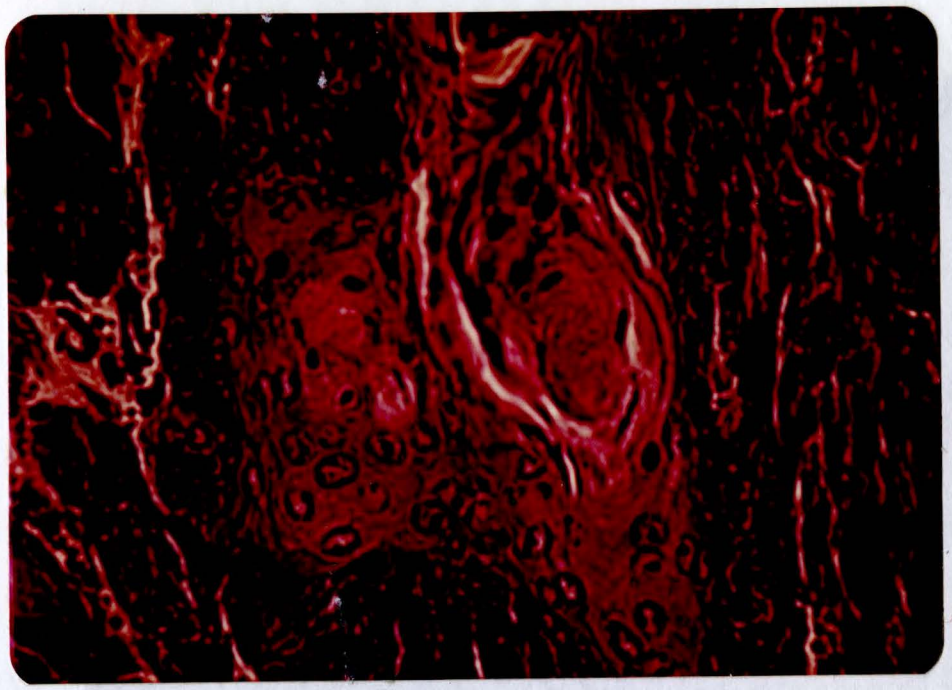


FIGURE 4

INVADING SQUAMOUS CELL CARCINOMA

Epithelial pearls. Infiltrating nests of squamous epithelial cells. X400 H.+E.

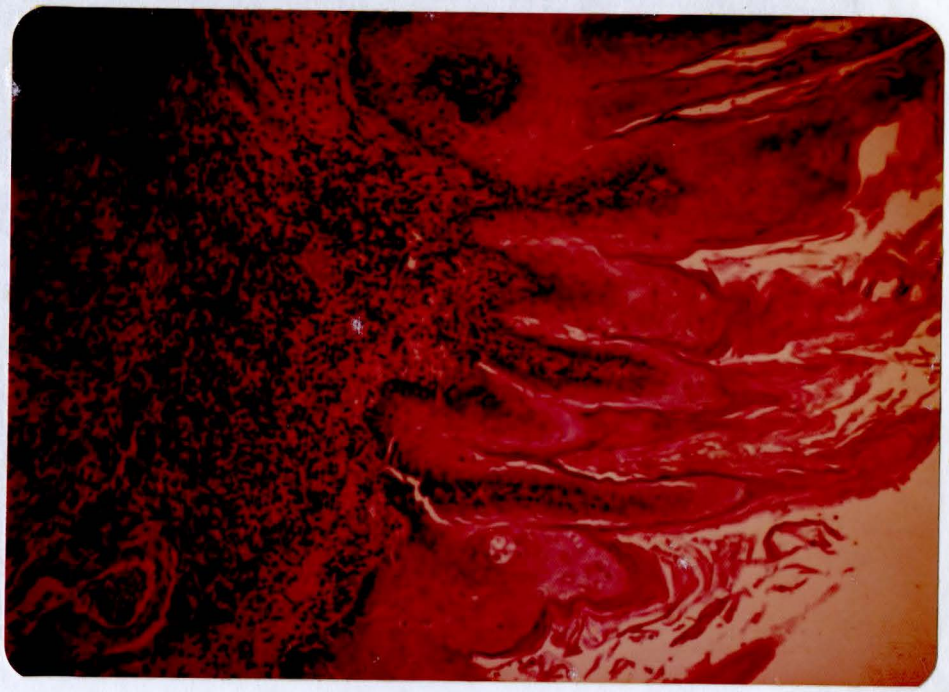


FIGURE 5

PAPILLOMA

X100 H.+E.

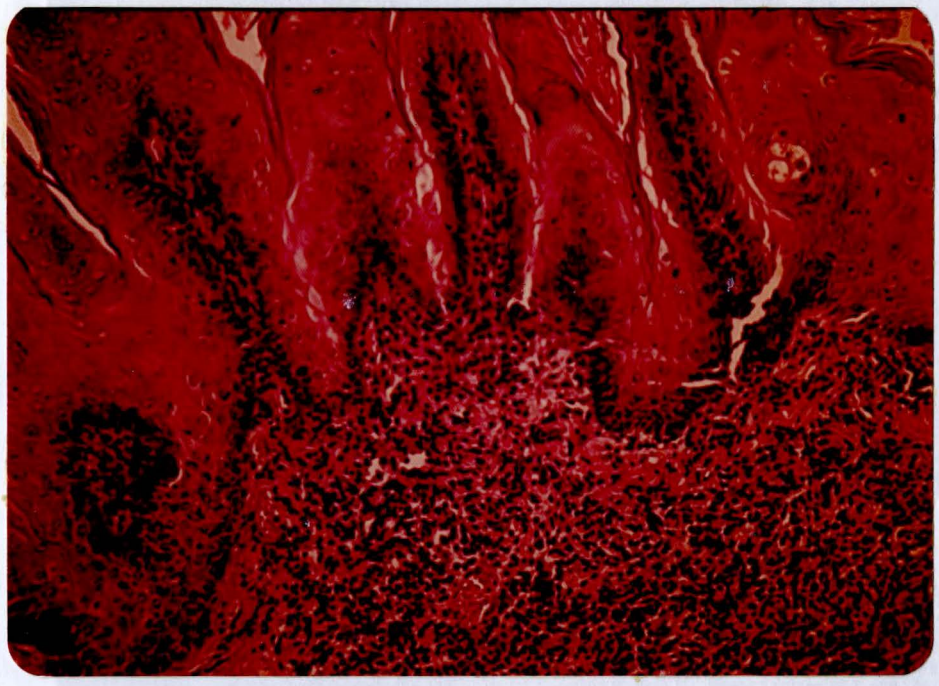
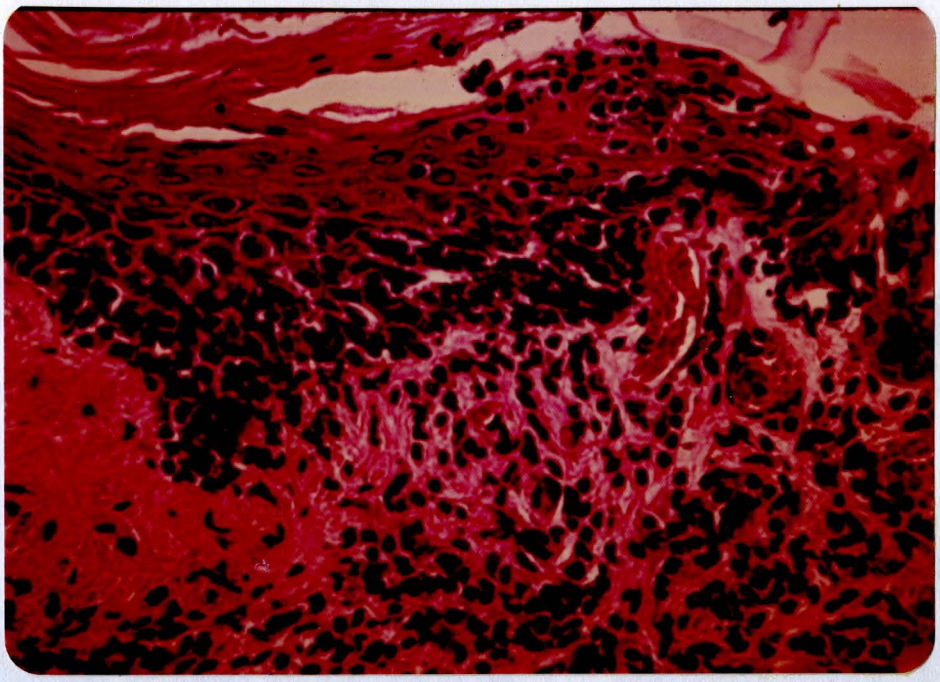


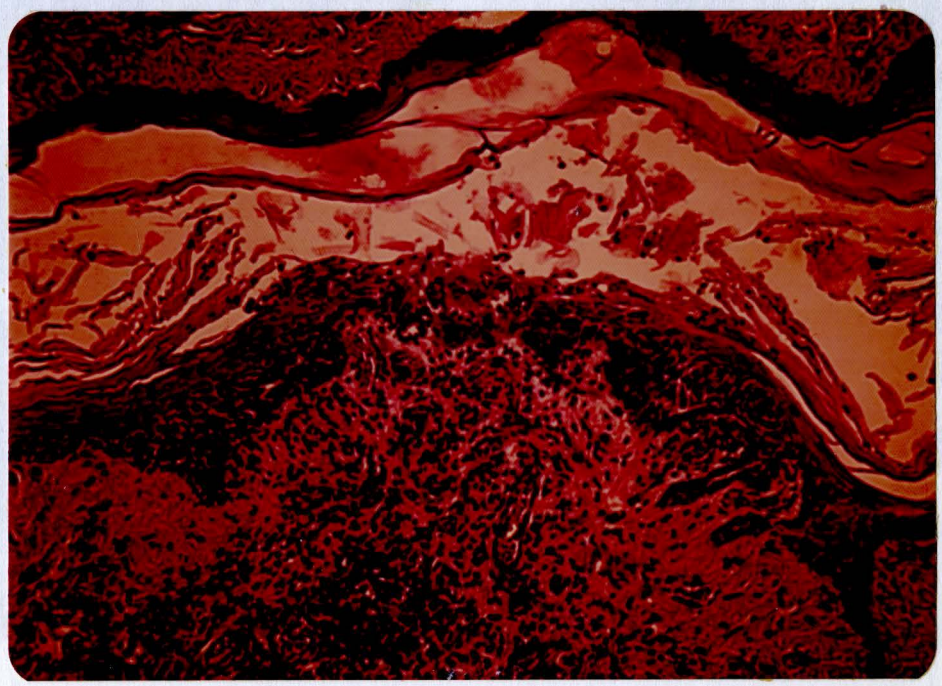
FIGURE 6

PAPILLOMA WITH LYMPHOCYTE INFILTRATION

X250 H.+E.



X400 H.+E.



X250 H.+E.

FIGURE 7

LYMPHOCYTES INVADING LAMINA PROPRIA IN ULCERATED AREA

FIGURE 8

LAMINA PROPRIA HAMSTER POUCH SHOWING CAPILLARY

AND DEVELOPING PLASMA CELL

ELECTRON MICROGRAPH
X16800

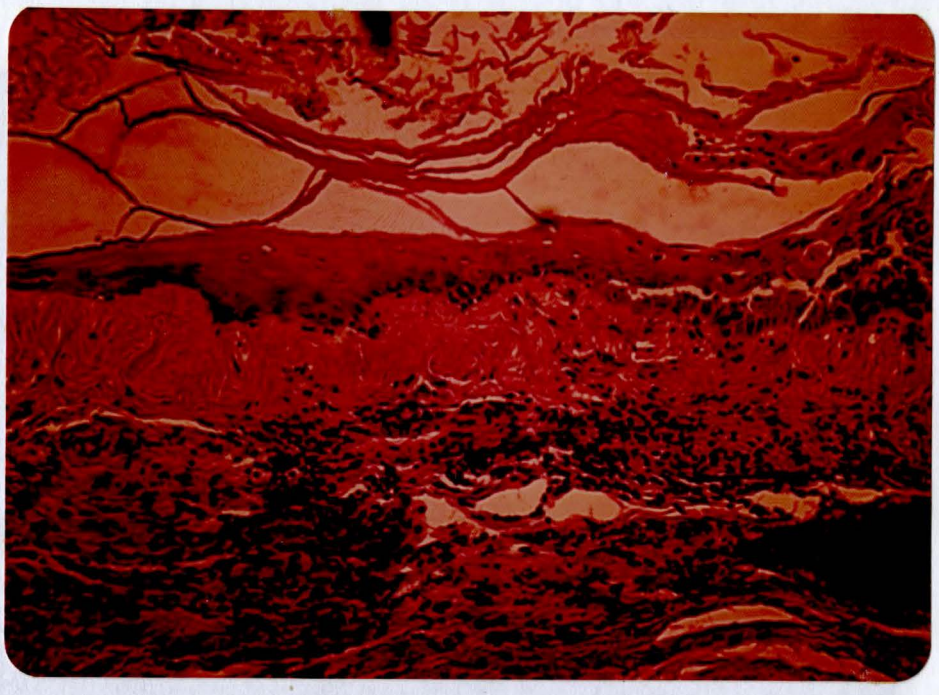


FIGURE 9

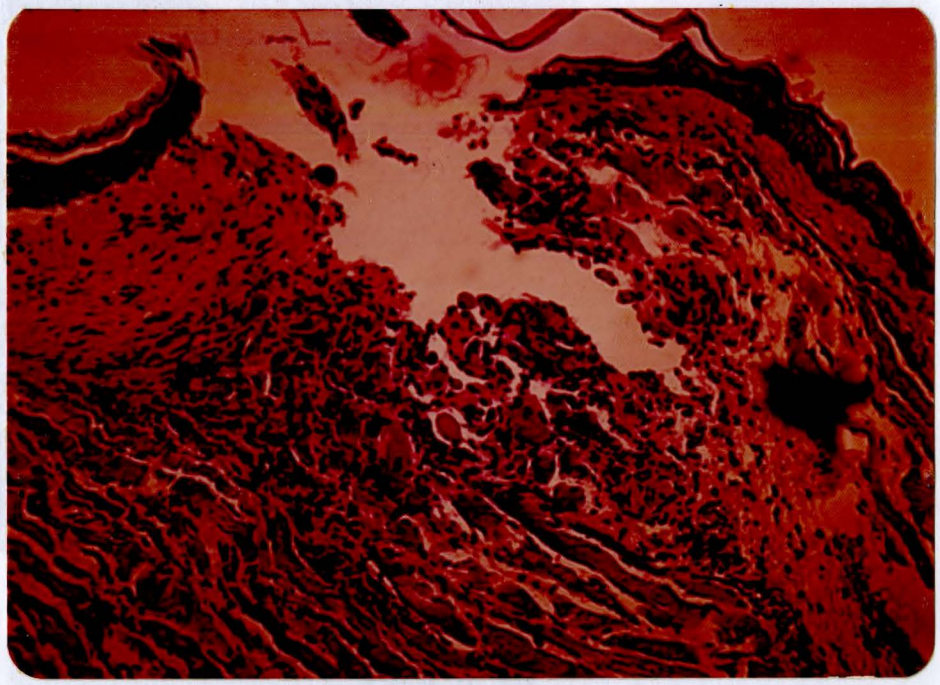
ACANTHOSIS WITH HYPERKERATOSIS AND
LYMPHOCYTIC INFILTRATION

X250 H.+E.

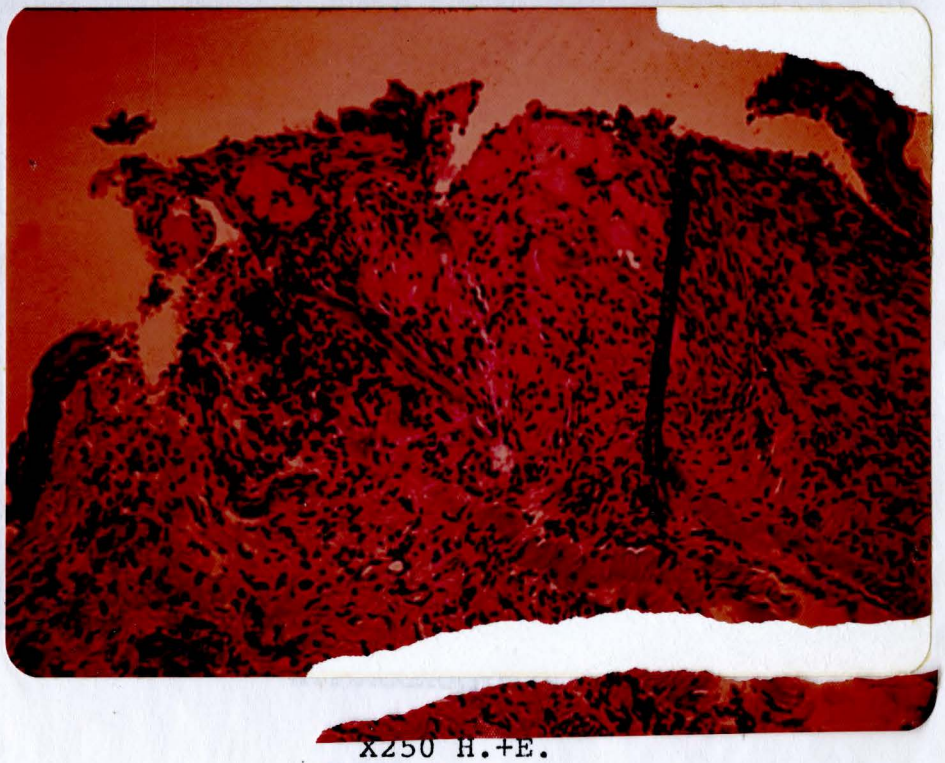
FIGURE 10

THICKENED EPITHELIUM WITH KERATIN PEELING OFF

X250 H.+E.



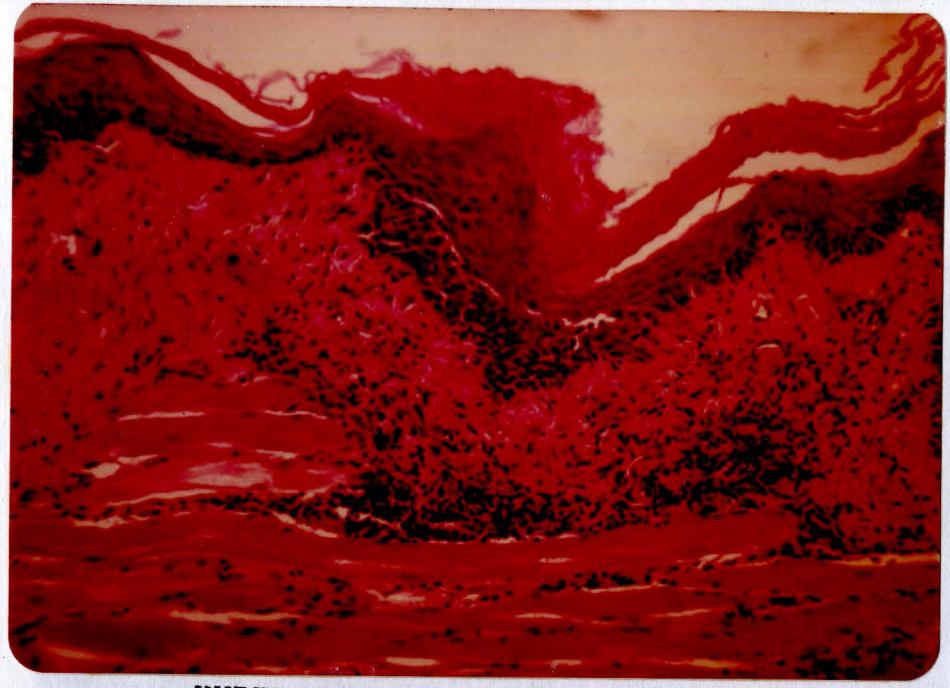
X100 H.+E.



X250 H.+E.

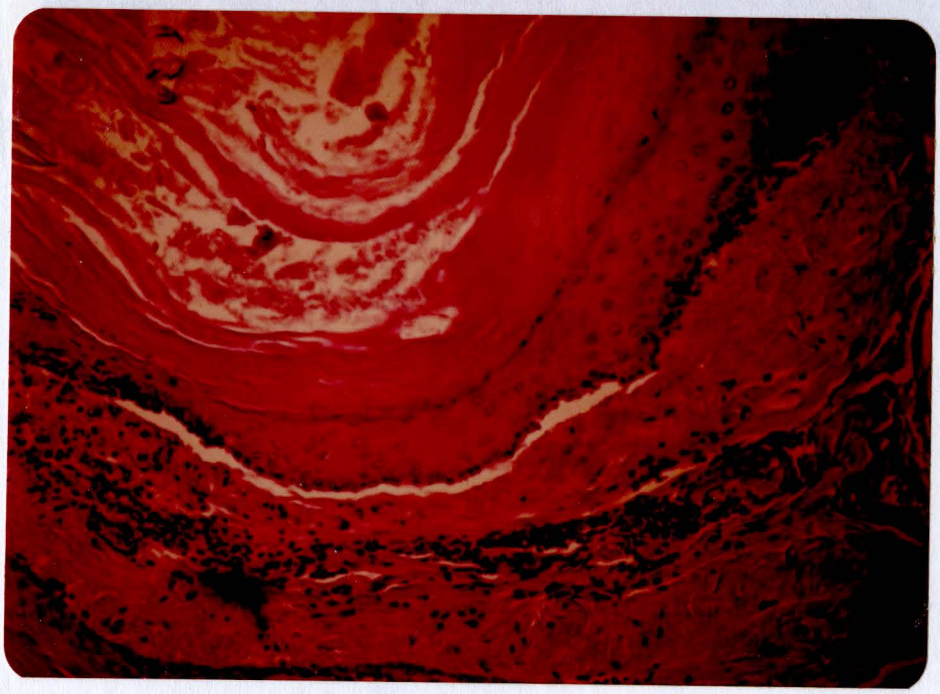
FIGURE 11

ULCERATION WITH LYMPHOCYTIC INFILTRATION



HYPERKERATOSIS WITH ACANTHOSIS

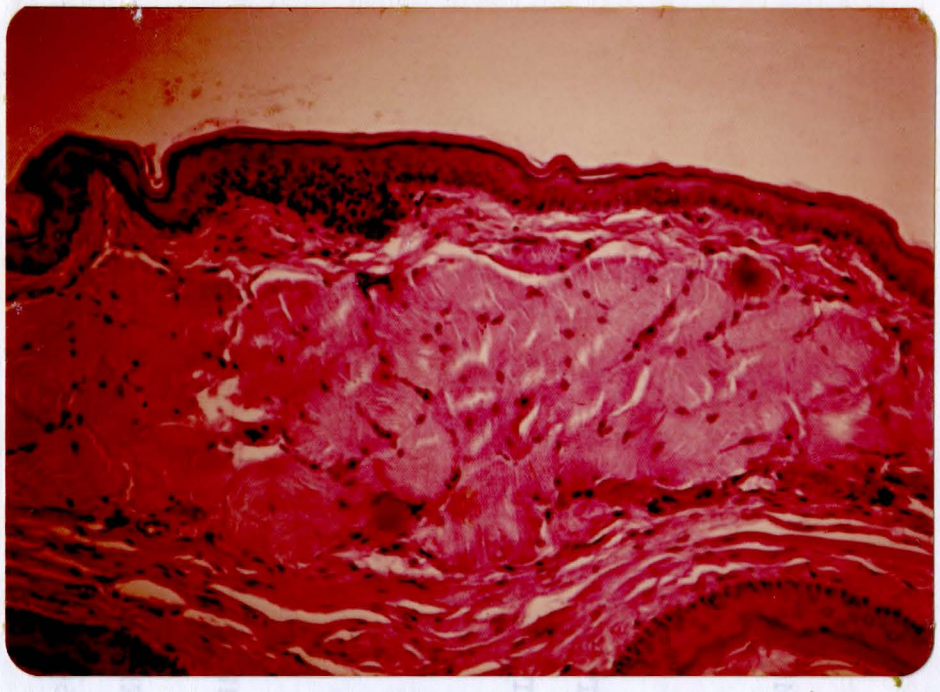
X250 H.+E.



HYPERKERATOSIS

X400 H.+E.

FIGURE 12



NORMAL HAMSTER POUCH EPITHELIUM

X100 H.+E.

TABLE I

DNCB CELL MEDIATED RESPONSE DETERMINATION

INITIAL SERIES

DAY 1 AND DAY 3 SENSITIZING DOSE

Hamster	0.1%	0.5%	1%	5%	10%
1	Left Pouch	Right Pouch			
2	L.P.	R.P.			
3			L.P.	R.P.	
4			L.P.	R.P.	
5					L.P.
6					L.P.
	Clinically Normal	Clinically Normal	Necrotic	Necrotic	Necrotic

TABLE I continued

DAY 17 CHALLENGING DOSE

Hamster	.01%	.05%	.1%	0.5%	1%
1	L.P.	R.P.			
2	L.P.	R.P.			
3			L.P.	R.P.	
4			L.P.	R.P.	
5					L.P.
6					L.P.
	Clinically Normal	Necrotic	Necrotic	Necrotic	Animal Died

TABLE II

DNCB CELL MEDIATED RESPONSE DETERMINATION

SECOND SERIES

DAY 1 AND DAY 3 APPLICATIONS

Hamster	.000005%	.00001%	.0005%	.001%	.05%	0.1%	Acetone
1	LP RP						
2	LP RP						
3		LP RP					
4		LP RP					
5			LP RP				
6			LP RP				
7				LP RP			
8				LP RP			
9					LP RP		
10					LP RP		
11						LP RP	
12						LP RP	
13							LP_o RP_o
14							LP_o RP_o

\+ = Optimum Response
\- = Failure of Response
o = Control
LP = Left Pouch
RP = Right Pouch

TABLE II continued

DAY 17 APPLICATION

Hamster	.0000005%	.000001%	.00005%	.0001%	.005%	.01%	Acetone
1	LP- RP-						
2	LP- RP-						
3		LP- RP-					
4		LP- RP-					
5			LP- RP-				
6			LP- RP-				
7				LP- RP-			
8				LP- RP-			
9					LP- RP-		
10					LP- RP-		
11						LP+ RP+	
12						LP+ RP+	
13							LP_o RP_o
14							LP_o RP_o

+ = Optimum Response
- = Failure of Response
o = Control

TABLE III A

FLOWSHEET OF DNCB-DMBA EXPERIMENTS

Exp. I Group A	Day 1	Day 3	Day 17	Day 18	
Sensitization With DNCB Then DMBA (3 Animals)	.1% DNCB Sensitizing Dose	.1% DNCB Sensitizing Dose	.01% Challenging Dose	DMBA → Continue Biweekly Paintings For 14 Weeks	→ Sacrifice

TABLE III B

FLOWSHEET OF DNCB-DMBA EXPERIMENTS

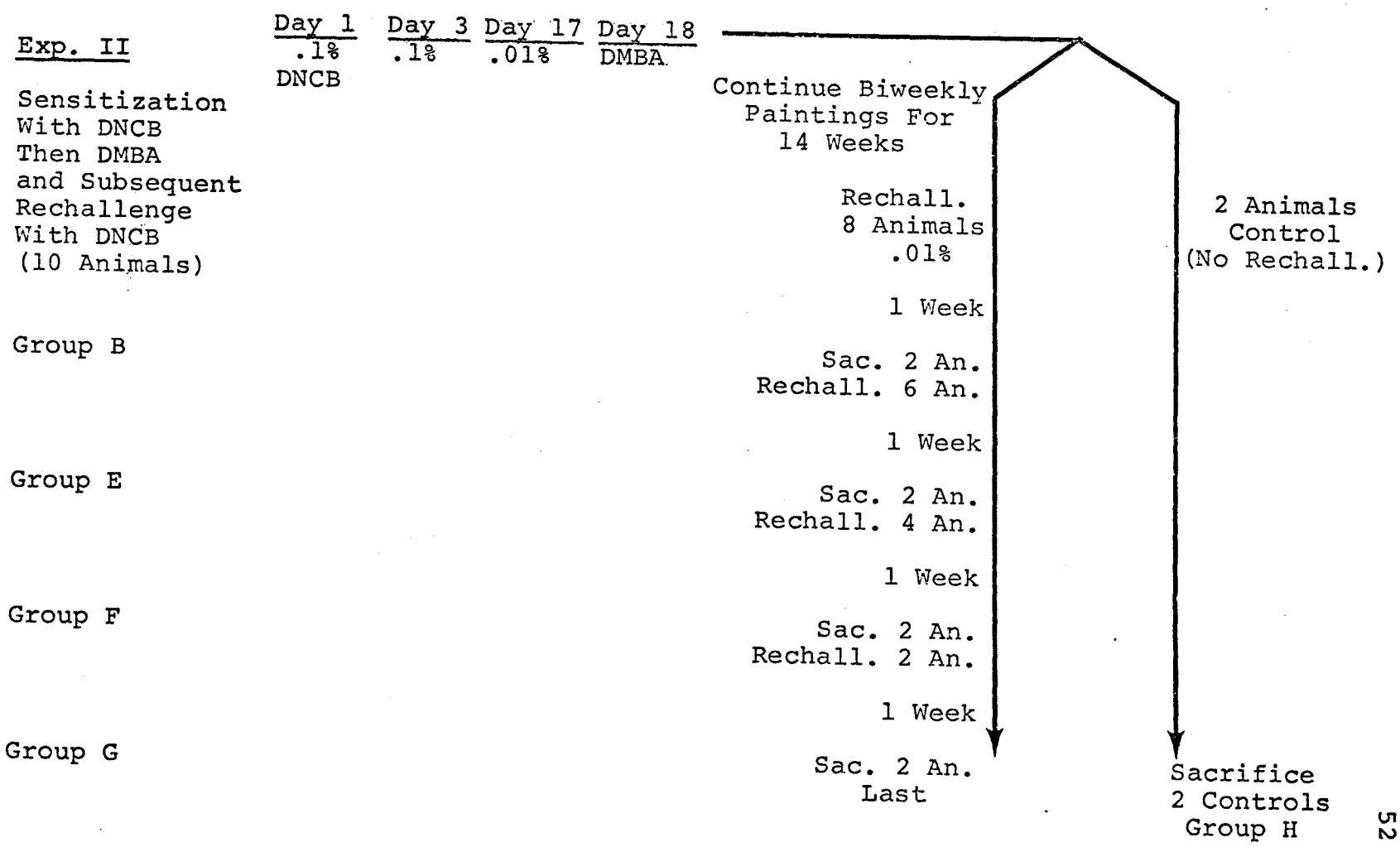


TABLE III C

FLOWSHEET OF DNCB-DMBA EXPERIMENTS

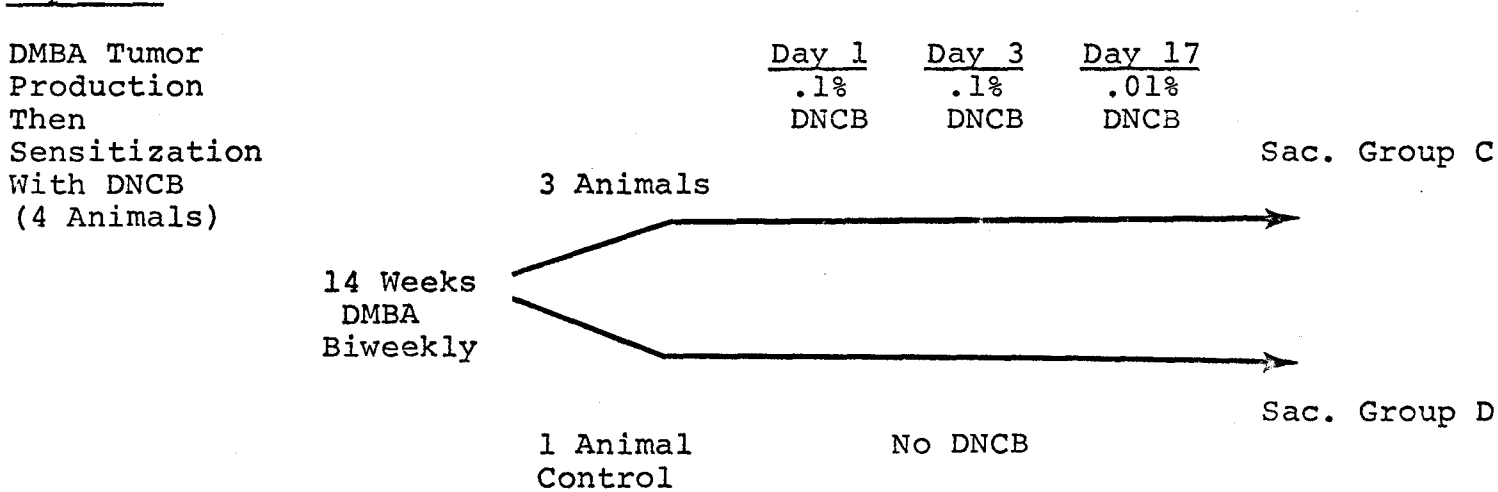


TABLE IV

DNCB-DMBA EXPERIMENT

Exp. I

Treatment
With DNCB
Followed
By DMBA

Group A	Tumor Type	Tumor Size μ	Other Epithelial Change	Lymphocyte Count/50 μ^2	Plasma Cell Count/50 μ^2
Pouch 1	None	None	1. Hyperplasia	20	.5
			2. Hyperplasia With Dyskeratosis		
Pouch 2	Squamous Ca. In Situ	50 X 200	None	"	"
Pouch 3	None	None	"	"	"
Pouch 4	Papilloma	40 X 120	"	"	"

TABLE V

DNCB-DMBA EXPERIMENT

Exp. II

Treatment With DNCB Followed By DMBA And Rechallenged With DNCB

Group B	Tumor Type	Tumor Size μ	Other Epithelial Changes	Lymphocyte Count/50 μ^2	Plasma Cell Count/50μ^2
Pouch 1	None	None	Hyperplasia With Hyperkeratosis	20	.5
Pouch 2	"	"	None	"	"
Pouch 3	"	"	"	"	"
Pouch 4	"	"	"	"	"

TABLE V continued

Group E	Tumor Type	Tumor Size μ	Other Epithelial Changes	Lymphocytes Count/50 μ^2	Plasma Cell Count/50 μ^2
Pouch 1	None	None	Hyperplasia With Hyperkeratosis	20	.5
Pouch 2	"	"	None	"	"
Pouch 3	"	"	"	"	"
Pouch 4	Papilloma	50 X 100	"	"	"
Group F					
Pouch 1	None	None	Hyperplasia With Hyperkeratosis	"	"
Pouch 2	"	"	Hyperplasia	"	"
Pouch 3	"	"	None	"	"
Pouch 4	"	"	"	"	"

TABLE V continued

Group G	Tumor Type	Tumor Size μ	Other Epithelial Changes	Lymphocyte Count/50 μ^2	Plasma Cell Count/50 μ^2
Pouch 1	None	None	Hyperplasia	20	.5
Pouch 2	Papilloma	100 X 175	None	"	"
Pouch 3	None	None	"	"	"
Pouch 4	"	"	"	"	"
Group H					
Pouch 1	Squamous Cell Ca. In Situ	300 X 400	Hyperplasia	"	"
	Squamous Cell Ca. In Situ	300 X 400	Hyperplasia	"	"
Pouch 2	None	None	None	"	"
Pouch 3	"	"	Hyperplasia	"	"
Pouch 4	"	"	None		

TABLE VI

DNCB-DMBA EXPERIMENT

Exp. III

DMBA Tumor Production Then DNCB Treatment

Group C	Tumor Type	Tumor Size μ	Other Epithelial Changes	Lympohcyte Count/50 μ^2	Plasma Cell Count/50 μ^2
Pouch 1	Papilloma	135 X 135	Ulcer	50	2.7
	"	40 X 80	"	"	"
	"	60 X 60	None	"	"
	"	60 X 60	"	"	"
Pouch 2	"	135 X 150	Ulcer	"	"
	"	110 X 110	None	"	"
	"	150 X 200	"	"	"

TABLE VI continued

	Tumor Type	Tumor Size μ	Other Epithelial Changes	Lymphocyte Count/50 μ^2	Plasma Cell Count/50 μ^2
Pouch 3	Papilloma	100 X 150	Hyperplasia	50	2.7
	Papilloma Or Carcinoma In Situ	300 X 200	None	"	"
	Papilloma	150 X 75	"	"	"
Pouch 4	"	50 X 50	Hyperplasia	"	"
	"	50 X 80	None	"	"
Group D (Control)					
Pouch 1	Invasive Carcinoma	450 X 250	None	12.2	.66
Pouch 2	Invasive Carcinoma	400 X 300	"	"	"

APPROVAL SHEET

The thesis submitted by Martin Marshack has been read and approved by the following committee:

Dr. Patrick D. Toto, Director
Professor and Chairman, Oral and General Pathology, Loyola

Dr. William Malone
Professor, Oral Biology, Loyola

Dr. Robert J. Pollock
Associate Professor, Oral Biology, Loyola

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

5-17-1976
Date

Patrick D. Toto
Director's Signature